

IN THE CLAIMS:

This listing of claims replaces all prior versions, and listings, of claims in the application:

1. (Currently Amended) A method for ~~in-vitro~~ constructing ~~of a~~ functional mammalian organ or a fragment thereof in vitro, comprising:

culturing and propagating embryonic epithelial-derived explants, tissue or cells by comprising:

isolating the tissues or cells and growing them in culture,

permitting the culture to form multiple branches, dissecting out ~~the~~ individual branch tips,

reculturing the individual branch tips in the presence of serum, a growth factor mix, conditioned medium and nutrient rich medium for several generations to generate branch tip buds;

simultaneously culturing and propagating isolated embryonic or fetal metanephric mesenchyme by comprising

dissecting out fetal mesenchyme at the time of induction,

culturing mesenchymal tissue in the presence of serum, a growth factor mix, conditioned medium and nutrient-rich medium, partitioning the mesenchyme into multiple pieces and growing each piece separately, and

inducing vasculogenesis by subjecting grown mesenchyme to substrate deprivation or addition or soluble factors;

recombining each vascularized mesenchyme with each cultured bud in a matrix in which in vitro angiogenesis has begun; and

growing ~~in richest medium~~ under conditions to ensure continued vasculogenesis.

2. (Withdrawn) A method for in vitro culturing and propagating ureteric bud tissue, comprising:

isolating embryonic kidney rudiments by dissection,
isolating ureteric bud tissue fragments from mesenchyme by
incubating said kidney rudiments with a proteolytic enzyme
in the presence of DNase and/or by mechanical separation;

suspending said isolated ureteric bud fragments in a gel
matrix;

placing the gel/fragment composition on porous
polycarbonate membrane inserts in wells of tissue culture
plates;

adding growth factors to the culture wells;

maintaining the gel composition at the interface of air and
medium until said fragments form multiple tubular branches
inside the gel matrix;

dissecting out distal individual branch tips formed during
culture; and

reculturing said branched tips in the presence of serum,
growth factor mix, cell conditioned medium and nutrient-rich
medium for several generations.

3. (Withdrawn) The method according to claim 2, wherein the
mechanical separation is accomplished by manual dissection.

4. (Withdrawn) The method according to claim 2, wherein the
mechanical separation is accomplished by laser separation and
capture.

5. (Currently Amended) The method according to claim ~~2~~ 1, wherein the growth factor mix comprises a glial cell line-derived neurotrophic factor or functional equivalent thereof

6. (Currently Amended) The method according to claim ~~2~~ 1, wherein the added conditioned medium comprise a growth promoting constituent or inducer of differentiation or morphogenesis.

7. (Currently Amended) The method according to claim ~~2~~ 1, wherein the ~~extracellular~~ matrix ~~gel~~ comprise a mixture of type I collagen and Matrigen, or an equivalent matrix.

8. (Withdrawn) A method for in vitro culturing and propagation of metanephric mesenchyme, comprising:

dissecting out fetal kidney mesenchyme tissue [at the time of induction];

culturing said mesenchymal tissue in the presence of serum, growth factor mix, mesenchymal and/or bud cell conditioned medium and nutrient-rich medium;

partitioning the cultured mesenchyme into multiple pieces and growing each piece separately in culture; and

subjecting grown mesenchyme to substrate deprivation or addition of vasculogenic growth factor sin order to induce vasculogenesis.

9. (Withdrawn) A method for in vitro engineering and constructing a mammalian kidney, comprising:

culturing and propagating a ureteric bud by

isolating the ureteric bud in culture,

permitting the culture to form multiple branches,
dissecting out the individual branch tips,
reculturing in the presence of serum, growth factor mix,
mesenchymal and/or bud cell conditioned medium and
nutrient-rich medium for several generations;

culturing and propagating isolated embryonic or fetal
metanephric mesenchyme by

dissecting out fetal mesenchyme at the time of
induction,

culturing mesenchymal tissue in the presence of serum,
growth factor mix,

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conditioned medium and nutrient-rich medium,

partitioning the mesenchyme and growing each piece
separately, and

inducing vasculogenesis by subjecting grown mesenchyme
to substrate

deprivation or addition of vasculogenic growth factors;

recombining each vascularized mesenchyme piece with
each cultured bud in a matrix in which in vitro angiogenesis has
begun; and

growing in richest medium conditions to ensure
continued vasculogenesis.

10. (Currently Amended) The method according to claim 9 1,
wherein the ~~recombined~~ tissues are implanted into a recipient
without prior induction of vasculogenesis.

11. (Withdrawn) A function mammalian kidney constructed in
vitro from isolated embryonic or fetal kidney tissue or cells

that are cultured in rich medium having present a mixture of growth factors and inducer substances, comprising:

an isolated ureteric bud propagated in culture to produce a functioning nephron;

metanephric mesenchyme propagated from cultured embryonic mesenchymal tissue fragments or cells; and

recombination of propagated ureteric bud and metanephric mesenchyme wherein said recombination in culture results in a functioning kidney or a functionally equivalent fragment thereof.

12. (New) The method of claim 1, wherein the tissue is mammalian kidney tissue.